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'Molecular sandwiches' as a basis for structural and functional similarities of interferons, MSH, ACTH, LHRH, myelin basic protein, and albumins

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Sequential similarities between the tryptophan peptide of myelin basic protein (residues 111–121), luteinizing hormone releasing hormone, melanotropin, adrenocorticotropin (residues 1–13), human leukocyte interferon (residues 28–40), and various segments of human and bovine serum albumin and hen ovalbumin are presented. It is suggested that these structural similarities may explain observations concerning common functional characteristics such as serotonin modulation, immunological activity with the adjuvant muramyl dipeptide, immunological cross-reactivity, and the possible MSH-ACTH-like activity of a pepsin-derived peptide of interferon.

MSH *ACTH* *LHRH* *Myelin basic protein* *Interferon* *Albumin*

1. INTRODUCTION

Authors in [1–3] have reported that anti-sera to α-melanotropic hormone (MSH) (which also corresponds to α-adrenocorticotropic hormone, residues 1–13 [ACTH (1–13)]) cross-reacts with human leukocyte interferon (αIFN). "Conversely, anti-sera to human leukocyte interferon neutralized ACTH activity." They further claim that an unidentified pepsin-derived peptide from αIFN possessed ACTH-like activity. Anti-sera to MSH-ACTH (1–13) did not cross-react with fibroblast interferon (βIFN). No cross-reactivity was observed between anti-sera to luteinizing hormone (LH) or follicle-stimulating hormone (FSH) and human leukocyte interferon. Assuming a correlation between immunological cross-reactivity and structural similarity [4], these observations suggest that MSH-ACTH (1–13) and some portion of αIFN are structurally similar [5]. Authors in [5] and in [6] have both reported that no significant degree of homology exists between ACTH and αIFN, and the latter authors [6] have not been able to replicate the results of those in [1–3]. However, they did not use the same system as that in [1–3] for testing the reported results, nor did they test for ACTH-like activity of a peptide fragment of αIFN. Thus, while there is some question as to the validity of the results in [1–3], it was deemed of sufficient interest to take another look at the possibility of a structural similarity between MSH-ACTH (1–13) and αIFN. A sequential similarity was, in fact, found, and, in the process, similarities between ACTH and αIFN were found with a number of other peptides and protein sequences.

2. METHODS

The method used for locating the sequential similarity is based upon theoretical and experimental considerations of the active sites of peptide hormones. Authors in [7] have suggested that active sites of peptide hormones usually, if not always, contain one or more aromatic residues. The author in [8] has suggested further that pairs of aromatic residues at active sites form 'molecular sandwiches' comprised of two aromatic residues separated by a hydrophilic one that can intercalate

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aromatic amines such as serotonin (5-hydroxytryptamine [5HT]), dopamine, and histamine. Authors in [9,10] located one such 5HT-binding site on the tryptophan peptide of myelin basic protein (residues 111–121) (table 1) that contains a molecular sandwich [8]. It was then demonstrated by means of NMR spectroscopy that the molecular sandwich model of 5HT binding to tryptophan peptide agrees with observed modes of interaction and that similar molecular sandwiches exist on MSH, ACTH, and luteinizing hormone-releasing hormone (LHRH) (table 1) [11]. It has been shown further that the proposed molecular sandwiches correspond to the known active sites of MSH, ACTH, and LHRH [12–16]. The report in [1–3] of cross-reactivity between MSH-ACTH (1–13) and human leukocyte interferon correlated with the ACTH-like activity of a human leukocyte

Table 1

Search for sequence homology to the molecular sandwiches of MSH, ACTH, LHRH, and tryptophan peptide

Myelin basic protein (111-121)	Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg
LHRH	< Glu-His-Trp-Ser-Tyr-Gly-Glu-Arg-Pro-NH_2
ACTH-MSH (1-13) (Phe substitution active)	(Phe) Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val
Human leukocyte interferon (α) (28-40)	Ser-Cys-Leu-Met-Asp-Arg-His-Asp-Phe-Gly-Phe-Pro-Gln
Human fibroblast interferon (β) (28-40)	Ser-Cys-Leu-Lys-Asp-Arg-Met-Asn-Phe-Asp- Ile -Pro-Glu
Human serum albumin (5-15) (bovine identical)	Ser-Glu-Val-Ala-His-Arg-Phe-Lys-Asp-Leu-Gly
Human serum albumin (330-337) (bovine in parentheses)	(Ser) (Ser) Met-Phe-Leu-Tyr-Glu-Tyr-Ala-Arg-Arg
Human serum albumin (503-512) (bovine in parentheses)	(Asp-Glu-Lys-Leu) Asn-Ala-Glu-Thr-Phe-Thr-Phe-His-Ala-Asp
Chicken ovalbumin (94-104)	Asn-Asp-Val-Tyr-Ser-Phe-Ser-Leu-Ala-Ser-Arg

The sequences (top to bottom) of the tryptophan peptide of myelin basic protein [36]; luteinizing hormone-releasing hormone [13]; melanotropic hormone (MSH) (which corresponds to adrenocorticotropic hormone, residues 1–13 [ACTH (1–13)]) [12]; human leukocyte interferon IFN-α1, residues 28–40 [17]; human fibroblast interferon βIFN [17]; human and bovine serum albumin [21,22]; and hen ovalbumin [23]. The active sites (molecular sandwiches [8]) of tryptophan peptide, LHRH and MSH-ACTH (1–13) [11–16] are double underlined. Note that Phe but not Ile may replace Trp without loss of activity in MSH-ACTH (1–13) [12]. These sequences are known to bind serotonin, and in the case of MSH-ACTH (1–13), histamine as well [11]. The molecular sandwich of human leukocyte interferon is also double underlined. Note that it retains the same components and structure of the other molecular sandwiches although differing in absolute sequence. Other homologies between MSH-ACTH (1–13) and tryptophan peptide, LHRH, human leukocyte interferon (28–40), and human fibroblast interferon (28–40) are indicated by single underlines. Note that while human fibroblast interferon (28–40) has many shared residues with human leukocyte interferon (28–40), it lacks a molecular sandwich and has a few other similarities to MSH-ACTH (1–13). The albumins also contain molecular sandwiches similar to the 5HT-binding sites of tryptophan peptide, LHRH, and MSH-ACTH. As noted in the text, no molecular sandwiches were found on follicle-stimulating hormone or luteinizing hormone [19], nor were 5HT-binding sites found on delta sleep inducing peptide, gastrin, somatostatin, enkephalin, or proctolin [11]

interferon peptide then suggested that interferon might also possess an active site composed of a molecular sandwich similar to the 5HT-binding sites already elucidated. A search of the interferon sequence [17,18] was therefore made for a sequence homologous to the molecular sandwiches of MSH, ACTH, LHRH, and tryptophan peptide.

3. RESULTS

The results of the search are shown in table 1. Only one molecular sandwich bearing any structural similarity to the 5HT-binding site of MSH-ACTH (1–13) was found on human leukocyte interferon: residues 33–36. Like previous molecular sandwiches, it consists of two aromatic residues separated by a hydrophilic residue. The human leukocyte interferon 'sandwich' sequence, while not identical to the ACTH sequence, is composed of functionally similar residues. The amino acid composition of the sandwich is, in addition, very similar to that of ACTH, and the sandwich is flanked by 5 further residues within the sequence 28–40 that are identical to those in similar positions flanking the ACTH sandwich. In all, 8 of the 13 residues (28–40) in human leukocyte interferon are functionally analogous or identical to residues appearing in the same positions in MSH-ACTH (1–13). Because some of the residues are only functionally analogous and not identical, the similarities in sequence that I have proposed could not have been found by authors in [5] or [6] who looked for identities.

Human fibroblast interferon (β type) differs significantly from human leukocyte interferon [17] in the region of suggested homology to MSH-ACTH (1–13). In the first place, the molecular sandwich is absent: the His at position 34 is replaced with Met. Four other residues are substituted with functionally different amino acids, and one other (Gln for Glu at position 40) residue is also altered. This leaves only 3 of the residues held in common between human leukocyte interferon and MSH-ACTH (1–13) unaltered. No other ACTH-like homologies were found.

Only one possible molecular sandwich was found on αFSH (residues 84–92) and αLH (residues 82–90), and it is a sequence common to both: Cys-Ser-Thr-Cys-Tyr-Tyr-His-Lys-Ser [19]. Except for the existence of a pair of aromatic residues separated by a hydrophilic one (Tyr-Tyr-His), this sequence bears no similarities whatever to MSH-ACTH (1–13) or to human leukocyte interferon (28–40). The only possible molecular sandwich found on βFSH was a sequence in which a Cys separated two Tyr [18]. This Cys is involved in forming a disulfide bridge in the natural conformation and so is unavailable to participate as part of an active site [20]. In any case the sequence of which these residues are a part (28–36) is not homologous to MSH-ACTH (1–13) or human leukocyte interferon (28–40). No molecular sandwiches were found on βLH [19]. Experiments have demonstrated that no 5HT-binding sites exist on delta sleep-inducing peptide, gastrin, somatostatin, enkephalin, or proctolin [11]. Several molecular sandwiches similar to those of ACTH, LHRH, etc., were found, however, on human serum albumin [21], bovine serum albumin [22] and chicken ovalbumin [23] (table 1).

4. DISCUSSION

The proposed homology between MSH-ACTH (1–13) and human leukocyte interferon (28–40) is consistent with a large number of observations. It explains the observed cross-reactivity between MSH-ACTH (1–13) anti-sera and human leukocyte interferon and vice versa; the lack of cross-reactivity between MSH-ACTH (1–13) anti-sera and human fibroblast interferon; and the lack of cross-reactivity between anti-sera to LH and FSH with human leukocyte interferon [1]. It also accounts for the MSH-ACTH-like activity of a pepsin-derived peptide of human leukocyte interferon [1], since the proposed sequence contains an active site (molecular sandwich) similar to that previously identified on MSH-ACTH [12–16]. Indeed, it is known that a tryptic digest of leukocyte interferon yields a peptide composed of residues 32–45 [24]. Since this peptide contains most of the homologous region including the active site, it may have MSH-ACTH-like activity.

The proposed homology leads to several other testable predictions. MSH, ACTH, and LHRH activities are modulated by 5HT [25–28], and the molecular sandwiches on these hormones and tryptophan peptide act as 5HT-binding sites [10,11]. The albumins are also known to bind 5HT [29–33]. It follows that the molecular sandwich on

human leukocyte interferon may also act as a 5HT-binding site, and thus that 5HT may also modulate interferon activity. Model building with CPK models suggests the further possibility that, like MSH and ACTH [11], the molecular sandwich on human leukocyte interferon may bind histamine. Since 5HT and histamine are integrally involved in immunological reactions of many kinds [34,35], their modulation of interferon activity would not be unlikely. It is also noteworthy that the tryptophan peptide of myelin basic protein and LHRH are known to cause experimental autoimmune diseases in the presence of muramyl dipeptide [36–39], which has been shown to be a 5HT-like substance [40,41]. Muramyl dipeptide is also functional as an adjuvant for bovine serum albumin and hen ovalbumin [42,43]. These observations suggest a correlation between 5HT-binding sites and muramyl dipeptide activity [44] that may be extended to include MSH, ACTH, and αIFN. These predictions may be tested without great difficulty by Merrifield solid-phase synthesis of the relevant peptide sequences (table 1).

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